Differential Consumption of Coagulation Factors 
Resulting from Activation of the Extrinsic 
(Tissue Thromboplastin) or the Intrinsic 
(Foreign Surface Contact) Pathways

By H. L. Nossel

TWO MECHANISMS, the extrinsic and the intrinsic, have been defined 
whereby in vitro blood coagulation may be initiated. The extrinsic system 
is initiated by tissue thromboplastin which, in the presence of Factor VII and 
calcium, activates Factor X (Stuart-Prower factor). The intrinsic system 
is initiated by foreign surface contact which results in sequential activation of 
Factors XII (Hageman factor), XI (PTA-Plasma thromboplastin antecedent), 
and IX (Christmas factor or PTC-plasma thromboplastin component). 
The extent to which these activation mechanisms function in vivo is unknown 
and methods are required for identifying the occurrence of the different 
reactions which initiate the two pathways.

Previous studies have shown that activation of Factors XI and IX by foreign 
surface contact is associated with directly proportional consumption of these 
factors, and that serum derived from blood clotted with tissue thrombo-
plastin has a low Factor X content. The consumption of coagulation Factors 
IX, X, and XI resulting from activation of the intrinsic and extrinsic pathways 
was studied to determine whether the two activation mechanisms result in 
different patterns of coagulation factor utilization. The results of these studies 
are reported in this paper.

MATERIALS AND METHODS

Barbital buffered saline (pH 7.40), tissue thromboplastin (saline extract of human brain, 
approximately 4 mg./ml.), and phospholipid were made as described by Biggs and Mac-
farlane. Celite 512 (Johns-Manville) and aluminium hydroxide gel (Cutter) were used. 
Russell's viper venom (Burroughs Wellcome) was used in a 1/100,000 dilution, thrombofax 
(Ortho) was used undiluted and Factor X deficient plasma of bovine origin was supplied 
by Diagnostic Reagents (Thame, Oxon). Factor IX, X, and XI deficient plasma samples 
were obtained from patients with congenital deficiency of these factors. Substrate plasma 
for the thromboplastin generation tests was outdated blood bank plasma.

Blood for Consumption Studies

Blood for consumption studies was collected from normal subjects via a #20 gauge 
needle into a 35 ml. plastic syringe (Roehr) and added in 2 ml. aliquots to 10 × 75 mm.

From the Department of Hematology, The Mount Sinai Hospital, New York, N. Y. 
This study was supported in part by USPHS Grant HE-08631 from the National 
Institute of Arthritis and Metabolic Diseases, and by the Albert A. List, Frederick Machlin, 
and Anna Ruth Lowenberg Funds.

First submitted May 9, 1966; accepted for publication July 21, 1966.

H. L. Nossel, M.B., Ch.B., D.Phil.: Assistant Professor of Medicine, College of Physic-
ians and Surgeons, Columbia University, and Assistant Attending Physician, Presbyterian 
Hospital, New York, N. Y.
tubes. The tubes had been coated twice with a 10 per cent solution of siliclad (Clay-Adams) and heated to 160 C. for one hour after each coating. Each tube contained 1 ml. of either celite suspended in saline or of thromboplastin diluted in saline. Immediately after adding the blood, the tube contents were thoroughly mixed by repeated inversion over parafilm, and placed in a waterbath set at 37 C. The tubes were tilted at 1 minute intervals till the blood had clotted, and 4 hours after coagulation the serum was transferred to another 10 × 75 mm. silicone treated tube. The tube was incubated at 37 C. for 16 hours and at 4 C. for 96 hours. The serum was then tested without delay or after storage at −30 C. Whenever plasma or serum were incubated, the tubes were tightly stoppered.

In each case the assay results on serum derived from blood clotted with celite or thromboplastin were recorded as a percentage of the level found in the serum derived from the blood-saline mixture.

**Clotting Factor Assays**

Factor X was measured as described by Denson, except that thrombofax was used in place of lecithin. Some of the sera were assayed by a method in which plasma from a patient with congenital deficiency of Factor X was used (kindly supplied by Dr. C. Merske). The two methods gave almost identical results.

**Factor IX.** A partial thromboplastin time method was unsuitable for measuring Factor IX since the presence of tissue thromboplastin in the test sample invalidates the results. Factor IX was therefore measured by a thromboplastin generation method as described by Biggs and Macfarlane.

The test serum was prepared as follows: 0.5 ml. Factor IX deficient plasma, 0.1 ml. test sample and 0.1 ml. 1/200 saline brain extract were added to a new (nonsilicone treated) 10 × 75 mm. tube and warmed to 37 C. 0.5 ml. 0.025 M CaCl₂ was added and the tube contents were mixed by inversion over parafilm. Sixty minutes after the mixture had clotted, the clot was removed and 0.5 ml. of the serum was added to 2 ml. saline in a new (nonsilicone treated) 10 × 75 mm. tube. After 60 minutes incubation at 37 C. the tube was transferred to an ice bath at 4 C., where it remained during the day’s testing. A series of dilutions of the test serum in saline were made immediately prior to testing. Factor IX deficient serum was prepared in exactly the same way as the test serum, 0.1 ml. saline replacing the test sample. The Factor IX deficient serum was used without further dilution.

Aluminium hydroxide adsorbed plasma was prepared by adding 0.1 ml. undiluted aluminium hydroxide to 1 ml. of fresh normal plasma. The mixture was inverted several times to ensure thorough mixing and incubated at 37 C. for 1 minute. The aluminium hydroxide was removed by centrifuging and the supernatant plasma added to 4 ml. saline to make a 1/5 mixture.

Incubation mixtures were made by sequentially adding to an untreated tube 0.1 ml. volumes of Factor IX deficient serum, dilution of test serum, aluminium hydroxide adsorbed plasma (1/5), phospholipid (1/200) and CaCl₂ (0.025M). The incubation mixtures were tested for prothrombin converting activity, and the results were calculated as described by Biggs and Macfarlane.

**Factor XI.** Both the partial thromboplastin clotting time and celite eluate methods proved sensitive to the presence of tissue thromboplastin in the test samples and an assay method based on the thromboplastin generation principle was devised. The test serum was prepared by mixing 0.1 ml. test sample, 0.5 ml. Factor XI deficient plasma, 0.5 ml. 1/200 thromboplastin and 0.5 ml. CaCl₂ (0.025M) in an untreated 10 × 75 mm. tube. The mixture was allowed to clot at 37 C. and incubated at that temperature for 60 minutes. The clot was removed and 0.5 ml. serum added to 2.0 ml. saline in an untreated 10 × 75 mm. tube. After 60 minutes incubation at 37 C., the tube was transferred to an ice bath at 4 C. The dilution of the test sample at this stage was 1/60, and further dilutions in saline were made immediately before carrying out each test. Factor XI deficient serum was prepared in exactly the same manner except that 0.1 ml. saline was used in place of the test sample. The Factor XI deficient serum was used without further dilution. The absorbed plasma was
Table 1.—Results of Testing 2 Samples for Factor XI Concentration by a Thromboplastin Generation Test Method

<table>
<thead>
<tr>
<th>Sample and Dilution Tested</th>
<th>Incubation Time (Minutes)</th>
<th>Average Clotting Time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Serum 30:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1920</td>
<td>30.2</td>
<td>28.7</td>
</tr>
<tr>
<td>1/960</td>
<td>22.2</td>
<td>23.4</td>
</tr>
<tr>
<td>1/480</td>
<td>17.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Serum 31:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/960</td>
<td>33.2</td>
<td>30.8</td>
</tr>
<tr>
<td>1/480</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>1/240</td>
<td>18.4</td>
<td>18.6</td>
</tr>
</tbody>
</table>

prepared by mixing 0.1 ml. aluminium hydroxide with 1 ml. Factor XI deficient plasma for 1 minute at 37 C. The aluminium hydroxide was sedimented by centrifugation and the supernatant plasma decanted into 4 ml. saline. Each of the constituents of the incubation mixture was kept in a waterbath at 4 C. An incubation mixture was made by sequentially adding 0.1 ml. volumes of Factor XI deficient serum, dilution of test serum, aluminium hydroxide absorbed plasma, phospholipid (1/200) and CaCl₂ (.025M) to an untreated tube in a 37 C. waterbath. At intervals, 0.1 ml. of the incubation mixture was added to 0.2 ml. 0.025M CaCl₂ in a clotting tube. 0.2 ml. prewarmed substrate plasma was immediately added to the clotting tube and the clotting time recorded. Preliminary testing was required in order to select dilutions of the test serum and incubation times for testing so that the substrate clotting times were in a range between 15 and 35 seconds and the clotting time for each dilution remained reasonably constant. The results were then calculated as described by Biggs and Macfarlane¹ for the Factor VIII assay. A typical set of results is recorded in Table 1 and an example of the method of calculation in Figure 1. The Factor XI levels assayed by the thromboplastin generation method in a series of plasma samples with known Factor XI content is shown in Figure 2. Tissue thromboplastin was added to some of the plasma samples and the assays were repeated. The results recorded in Figure 3 indicate that the addition of thromboplastin did not alter the Factor XI level measured and that the test was therefore not affected by the presence of thromboplastin in the test samples. A similar set of tests showed that the Factor IX assay was not affected by thromboplastin.

RESULTS

Coagulation Factor Activities of Serum and Plasma

In a preliminary series of experiments, comparison was made between the coagulation factor activities of plasma and of serum derived from blood clotted in silicone treated tubes. Factor IX, X, and XI activity was almost identical in the serum as compared with the plasma (Table 2).

Blood Coagulation in the Presence of Celite

When whole blood clotted in the presence of increasing quantities of celite, the serum showed a progressive decrease in Factor XI content, being practically free of Factor XI activity when the initial celite concentration was 5 mg./ml. (Fig. 4). Similarly, the Factor IX content of the serum showed a progressive reduction with increasing initial amounts of celite. In this case the serum was practically free of Factor IX activity when the initial celite concentrations was 2.6 mg./ml. (Fig. 4). In contrast, celite caused very little consumption
Fig. 1.—Calculation of Factor XI level of Serum 31 as a percentage of Serum 30. The results recorded in Table 1 are plotted on double logarithm paper, each point representing clotting time plotted against serum dilution. Straight lines parallel to one another are drawn through each set of points. The 2 lines are compared by projection to the superior abscissa. In the example given above, Serum 31 has 44 per cent of the activity of Serum 30.

Fig. 2.—Factor XI levels measured by thromboplastin generation test in a series of plasma mixtures of known Factor XI concentration. Plasma samples containing different concentrations of Factor XI were made by diluting normal plasma in Factor XI deficient plasma, the concentration in normal plasma being regarded as 100 per cent.

Fig. 3.—Factor XI levels measured by thromboplastin generation test in a series of plasma mixtures of known Factor XI concentration. The same procedure as in Figure 2 was carried out except that undiluted brain thromboplastin was added to all the mixtures containing 6 to 75 per cent Factor XI (0.1 ml. of undiluted thromboplastin to 0.3 ml. plasma) but not to the 100 per cent Factor XI sample.
ACTIVATION OF EXTRINSIC AND INTRINSIC PATHWAYS

Table 2.—Coagulation Factor Activities of Serum and Plasma

<table>
<thead>
<tr>
<th></th>
<th>Factor IX (%)</th>
<th>Factor X (%)</th>
<th>Factor XI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Serum</td>
<td>101.4</td>
<td>98.7</td>
<td>101.5</td>
</tr>
</tbody>
</table>

Two ml. whole blood was allowed to clot in a silicone-treated tube at 37 C. and incubated at that temperature for 4 hours. After separation from the clot, the serum was incubated in a fresh silicone-treated tube for 16 hours at 37 C. and for 96 hours at 4 C. The serum was then tested without delay or after storage at -30 C. The plasma was separated from an aliquot of citrated blood and was incubated in an identical manner and at the same time as the serum. The results are the average of 6 separate experiments. The dilution of plasma by citrate (5 parts plasma to 1 part citrate) was taken into account in calculating the results. The concentration of trisodium citrate in the citrated plasma was 6.66 mg./ml.

Table 3.—Effect of Intrinsic and Extrinsic Activation on the Consumption of Coagulation Factors

<table>
<thead>
<tr>
<th>Activating Mechanism</th>
<th>Coagulation Factor Activity in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marked Reduction</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>Factor XI and IX</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>Factor X</td>
</tr>
</tbody>
</table>

of Factor X, about 70 per cent of the initial Factor X activity being recovered when the celite concentration was 16.6 mg./ml. (Fig. 4).

Blood Coagulation in the Presence of Tissue Thromboplastin

Practically no consumption of Factor XI activity resulted from the coagulation of blood in the presence of thromboplastin (Fig. 5). Similarly, only trivial consumption of Factor IX activity occurred in blood clotted in the presence of thromboplastin (Fig. 5). In contrast, over 90 per cent of the Factor X activity was consumed in blood clotted with 33 per cent thromboplastin (Fig. 5).

Blood Coagulation in the Presence of both Thromboplastin and Celite

When blood was clotted in the presence of both celite and thromboplastin, the thromboplastin reduced the consumption of Factors XI (Fig. 6) and IX (Fig. 7).

The effects of intrinsic and extrinsic activation on the consumption of coagulation Factors IX, X, and XI are summarized in Table 3.

DISCUSSION

Enzyme activity is classically studied by measuring substrate transformation. Transformation involves both the formation of a new product (activation) and the disappearance of the substrate (consumption). Investigation of the coagulation system sequence has most commonly been made by studying activation, with the use of partially purified fractions. In whole blood, in contrast, identification of specific factor activation may be more readily made by studying consumption. It should be possible to test the serum immediately after fibrin
Fig. 4.—Factor IX, X, and XI content of serum derived from whole blood clotted in the presence of celite. Two ml. blood was added to celite suspended in 1 ml. saline. The celite concentration of the completed mixture is shown on the abscissa. Four hours after coagulation, the serum was separated and incubated at 37 °C. for 16 hours and at 4 °C. for 96 hours.

Fig. 5.—Factor IX, X, and XI content of serum derived from whole blood clotted in the presence of brain thromboplastin. Two ml. blood was added to 1 ml. brain thromboplastin. The concentration of thromboplastin in the completed mixture is shown on the abscissa. Thirty-three per cent thromboplastin is equivalent to approximately 1.3 mg./ml. The serum was separated 4 hours after coagulation and incubated at 37 °C. for 16 hours and at 4 °C. for 96 hours.

Fig. 6.—Factor XI content of serum derived from blood clotted with both celite and thromboplastin and with celite alone. The concentration of celite in the completed mixture is shown on the abscissa. The concentration of thromboplastin in the completed mixture was 33 per cent. Two ml. blood was added to celite suspended in 1 ml. saline or undiluted thromboplastin. The serum was prepared as described in the legend to Figure 5.

— celite suspended in saline.

x - - - x celite suspended in thromboplastin.

Fig. 7.—Factor IX content of serum derived from blood clotted with both celite and thromboplastin and with celite alone. The procedure was as described in the legend to Figure 6.

— celite suspended in saline.

x - - - x celite suspended in thromboplastin.
has formed, but activated products of coagulation are present immediately after clotting. At present, it is not possible to measure coagulation factor activity in the presence of the activated product of the factor and the prolonged incubation period was accordingly necessary to permit inactivation of the intermediate products. Because of the entirely different conditions used for allowing the blood to clot and in preparing the serum, the present results cannot be compared with previous studies of coagulation factor alteration resulting from coagulation.

With regard to the assay methods used, a thromboplastin (Russell’s viper venom) is used for the Factor X assay and the results are not affected by the presence of thromboplastin in the test sample. For the Factor IX assays the thromboplastin generation method described by Biggs and Macfarlane proved satisfactory. The Factor XI assay method based on the thromboplastin generation principle and newly described here was not influenced by the presence of thromboplastin. Congenitally deficient plasma with complete or almost complete deficiency of Factor XI is essential for this test.

In accordance with the idea that consumption of factors is directly related to degree of activation, the consumption of Factors IX and XI in the presence of celite was expected, since celite has been shown to activate Factors XII and XI and thereby cause Factor IX activation. The minor degree of Factor X consumption resulting from intrinsic activation may have been anticipated, since although Macfarlane and Ash found evidence of Factor X activation during intrinsic coagulation, the amount of Factor VIII present in normal plasma was insufficient to completely activate Factor X and consumption could be enhanced by the addition of materials with high Factor VIII activity. Recently, Niemetz has found that when whole blood clots in the presence of celite, Factor X consumption is enhanced by addition of cephalin or of bovine concentrates with high Factor VIII activity.

Tissue thromboplastin is known to activate Factor X; hence, consumption of Factor X in the presence of thromboplastin was to be expected and had, in fact, been previously observed. It is not clear why complete consumption did not occur. Factor X tests in which the Russell’s viper venom reagent was omitted showed the sera to have negligible clotting activity under such circumstances, suggesting that residual activated Factor X activity was minimal. The failure of thromboplastin to cause Factor IX or XI consumption suggests that these factors are not activated during extrinsic prothrombin activator formation, as suggested by Biggs and Nossal and by Rapaport et al.

The effect of thromboplastin in reducing the consumption of Factors IX and XI produced by celite (Figs. 6 and 7) implies that activation of these factors was diminished. A possible explanation for this finding is that the thromboplastin caused rapid formation of fibrin which enmeshed the celite, thereby permitting the celite only about 20 to 30 seconds to activate Factors XI and IX instead of approximately 180 seconds in the absence of thromboplastin.

Activation is usually accompanied by consumption, but loss of activity does not necessarily imply activation and may be due to adsorption without activation or to nonspecific inactivation. Activation may only be inferred when the agent investigated has been shown to cause directly related activation and
consumption of the specific factor. Celite has been demonstrated to have such
an effect on Factor XI in normal blood.\textsuperscript{13} Factor XI is also adsorbed to celite
and it is likely that adsorption and activation are very closely related. An eluate
from celite, previously exposed to normal plasma, has been demonstrated to
produce directly related activation and consumption of Factor IX.\textsuperscript{14} Thus when
an eluate from celite or celite mixed with normal blood consumes Factor
IX, activation may be inferred. Nemerson and Spaet observed that boiled
thromboplastin adsorbs Factor X while causing only slight activation.\textsuperscript{2} High-
speed centrifugation (40,000 \text{rpm}) was used to sediment the thromboplastin
and adsorbed Factor X, whereas in the present experiments 2000 \text{rpm} centrif-
ugation for 5 minutes was used to separate the serum which retained signifi-
cant thromboplastic activity.

Regarding the application of the findings described here, the differential
coagulation factor consumption resulting from intrinsic and extrinsic activation
may be applicable to the study of the occurrence of these activation mechanisms
in vivo. For example, a study of Factor IX, X, and XI levels in patients with
disseminated intravascular coagulation may reveal patterns suggestive of the
occurrence of either the extrinsic or the intrinsic activation pathway. Rapaport
et al.\textsuperscript{23} recently found a reduction of Factor X activity in the blood of rabbits
infused with thromboplastin.

Factor IX and XI consumption has been shown to have a precise direct
relation with degree of activation.\textsuperscript{13,14} It should be possible to study the acti-
vation of coagulation factors for which such a relationship is found to hold
true, by measuring consumption. Kinetic studies of the activation of such coag-
ulation factors during the clotting of whole blood may be possible. A further
possible application may be in the preparation of artificially deficient reagents
suitable for coagulation factor assays. For example, a mixture of celite eluate
and of serum derived from blood clotted with celite would provide a thrombo-
plastin generation test serum reagent deficient in Factor IX but containing
Factor X and XI activity.

**Summary**

1. The consumption of coagulation Factors IX, X, and XI was studied in
normal whole blood clotted with celite (intrinsic activation) or tissue thrombo-
plastin (extrinsic activation).

2. During these studies an assay method for Factor XI was developed which
was not influenced by the presence of tissue thromboplastin. The assay method
is based on the thromboplastin generation principle.

3. When blood clotted in silicone treated tubes, the serum and plasma con-
centrations of Factors IX, X, and XI were almost identical, indicating that little
consumption or activation of these factors had occurred.

4. In the presence of celite, coagulation Factors IX and XI are consumed,
whereas Factor X is consumed only slightly.

5. In the presence of tissue thromboplastin, Factor X is consumed, whereas
Factors IX and XI are not consumed.
6. In the presence of both celite and thromboplastin, the thromboplastin decreased the consumption of Factors IX and XI produced by celite.
7. The study of serum coagulation factor levels may provide evidence as to whether the coagulation process had been initiated by the intrinsic (foreign surface contact) or extrinsic (thromboplastin) pathways.

**Summario in Interlingua**

1. Le consumo de Factores coagulatori IX, X, e XI esseva studiate in normal sanguine integre coagulate con celite (activation intrinsec) o con thromboplastina tissular (activation extrinsec).
2. In le curso de iste studios, un methodo de essayage pro Factor XI esseva disveloppate le qual non esseva influentiate per le presentia de thromboplastina tissular. Le methodo es basate in le principio del generation de thromboplastina.
3. Quando sanguine se coagulava in tubos tractate con silicona, le concentrationes seral e plasmatic del Factores IX, X, e XI esseva quasi identic, lo que indica que nulle consumo e nulle activation de iste factores habeva occurrute.
4. In le presentia de celite, le Factores coagulatori IX e XI es consumite sed non Factor coagulatori X.
5. In le presentia de thromboplastina tissular, Factor X es consumite sed non Factores IX e XI.
6. In le presentia de celite e de thromboplastina, le thromboplastina reduceva le consumo de Factores IX e XI supportate per celite.
7. Le studio de nivellos seral del factores coagulatori pote provider evidentia permittente decidere si le processo coagulatori ha essite initiate per le circuito intrinsec (contacto a superficie alien) o extrinsec (thromboplastina).

**REFERENCES**

Differential Consumption of Coagulation Factors Resulting from Activation of the Extrinsic (Tissue Thromboplastin) or the Intrinsic (Foreign Surface Contact) Pathways

H. L. NOSSEL